Research

SOUTH DAKOTA STATE UNIVERSITY

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Winter 2013

Inside

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South Dakota expertise integral part of LANDSAT 8

Landsat 8's South Dakota roots run deep. Not only is the main repository for Landsat data in Baltic, but two SDSU scientists are part of the 2012-2017 Landsat Science Team.

Dennis Helder, associate dean of research for the College of Engineering, and David Roy, senior scientist at the Geological Information Science Center for Excellence, are part of a 22-member team of nationally and internationally recognized scientists and engineers.

"SDSU has some of the top expertise in the country in this field," says Thomas Loveland, Landsat Science Team leader and a senior scientist at the U.S. Geological Survey Center for Earth Resources Observation and Science, commonly known as EROS. Only one other university has two researchers on the Landsat team.

All Landsat data is housed at EROS, which has more than 600 employees and stores nearly 3.5 million Landsat images taken over the last 40 years. "It's now the longest running record of the Earth's surface," Loveland says. EROS also archives aerial photos dating back to the 1930s.

"There's no place bigger than EROS," says Loveland, as a single source of remote land sensing data. It opened in 1972 when the first satellite was launched.

Helder, Roy experienced team members

The Landsat Data Continuity Mission became Landsat 8 when the satellite launched in February at Vandenberg Air Force Base in California. Helder and Roy were there.

Helder's history with Landsat dates back to 1988 when, as part of his doctorate, he solved a problem that caused stripes in Landsat 5 images; Roy has worked on Landsat for six years.

As part of the science team, Helder calibrates the images from the satellite so scientists like Roy can extract useful information from the data. Through the NASA-funded, Web-enabled Landsat Data project, Roy and his colleagues then convert this raw data into a user-friendly format.

Images will be the best ever

Helder anticipates the images from Landsat 8 will be the best ones ever. "It is clearly the most advanced instrument in the Landsat series," he says. "It will have better capability to resolve features on the Earth's surface."

When Helder calibrates the images, he turns them into data sets; each pixel measures the amount of energy welling up from the Earth at that location. "If we don't calibrate the images, they are only pretty pictures."

The dots that form an image are called pixels. In the past, each of those pixels was coded with eight bits, so it could be given a number from zero to 255. The Landsat 8 images have 12-bit coding, with numbers ranging from zero to 4,095.

That means the bright areas like clouds or snow and the dark areas like water will be more visible. "By making the steps sizes smaller, we can see the details better," Helder says.

The same is true when it comes to the signal-to-noise ratio. For example, a researcher can see small changes in plants, Helder says, "and still be assured that what he's seeing is the truth as opposed to the instrument being noisy."

In addition, unlike past satellites, Landsat 8 will collect images continuously, Helder says. Previous models had to be turned off at regular intervals because they got too hot.

"It can be on all the time," Helder says, opening up more opportunities to capture data. The satellite then downloads each time it flies over the receiving station.

All this is music to Roy's ears.

Free data easy to access

"Landsat is a crucial document of the Earth, and it's the only one," says Roy, who was elected co-chair of the Landsat Science Team last fall.

Loveland agrees, "From a global perspective, Landsat is the gold standard of Earth records."

Thanks to Roy's work, all the data over the United States are accessible to everyone free of charge at http://weld.cr.usgs.gov. The website that began in 2010 is simply the visual interface, Roy explains. More than 100,000 Landsat images are archived in his lab.

Each year more than 3 million Landsat images go to scientists and policymakers in more than 180 countries, making it one of South Dakota's biggest exports. Since they're now free, Loveland estimates that 70 percent of the archived images are being used, many of them repeatedly.

This wasn't always the case. Prior to 2008, scientists had to purchase these images for anywhere from $600 to $4,000 per image, and then had to manually work out the calibration and apply the algorithms necessary to extract the information.

"One scene might take half a day," Roy says. "Now this is all done with one click. Put your cursor on a point on the screen and in five minutes, you can grab the pixel values and plot the vegetation in a specific location every week for nine years."

The website contains nine years of data beginning from 2003 covering the continental United States and Alaska. The data can also be broken into specific seasons or months.

Roy says one survey suggests that the economic benefits of Landsat data are worth $7.5 billion per year. Using Landsat data, scientists and policymakers can map the sprawl of a new housing development or look at the influence of climate over long periods on the timing of the start of spring.

"The only global multi-decadal baseline for change is Landsat," Roy says. "In a period of time when the population has doubled and the evidence for climate change is increasing, it is so crucial that this record continues."

Dennis Helder

David Roy

Thomas Loveland

Internet users can now access images free of charge, whether those images are of the state of South Dakota in 2008 or the entire world in 2010, thanks to the work of the NASA-funded, Web-enabled Landsat Data project.

Morrill, Hatch Acts create educational opportunities, research enterprise

In 2012, SDSU celebrated two transformational events, the 150th anniversary of the Morrill Act of 1862 that in the midst of the Civil War expanded higher education opportunities and the 125th anniversary of the Hatch Act of 1887 that integrated research and scholarship into the enduring fabric of American higher education.

Although the land-grant institutions were charged with developing curriculums in agriculture and mechanic arts, their primary and lasting mission is to provide educational opportunities for common people, the so-called industrial classes. This fundamental mission of the Morrill Act was reinforced by subsequent legislation in 1890 and 1994 that targeted under-served minorities. The legacy of the Morrill Act lies in the opportunities that it created for all Americans to get a college education.

In 1887, the Hatch Act created state agricultural experiment stations, formalizing the research mission for land-grant institutions and putting agricultural research and education on the path to science-based discovery and innovation.

In its assessment of doctorate-granting universities nationwide, the Carnegie Foundation for the Advancement of Teaching designated SDSU as a high-research activity institution. Because of this commitment to research and scholarship, South Dakota's industries and communities have benefited from advances in technologies and a highly skilled workforce. As SDSU's research enterprise evolves, specific qualities are beginning to emerge.

First, SDSU's research is increasingly collaborative involving faculty from a diverse range of disciplines. Multidisciplinary teams are evolving into more tightly integrated interdisciplinary communities that bring about further synergies. As evidenced by the leadership roles that SDSU scientists and engineers have taken in Landsat 8, collaborative research with private industry, federal agencies, and other universities has increased.

Secondly, student participation is clearly a key to success. The SDSU Honors College has become a conduit for student engagement in research and scholarship. These enhanced opportunities for undergraduate students expand their horizons and build their self-confidence.

Finally, the research enterprise has become a driver of entrepreneurship, economic development and innovation, spawning new businesses that provide expanded employment opportunities. The university's technologies and inventions have been licensed to companies and are being commercialized. Research, such as the West Nile virus project, can have an immediate impact on the lives of South Dakotans.

Building on the recognition set forth 125 years ago that research and scholarship are essential for higher education, South Dakota State will continue its commitment to a research enterprise that benefits students and citizens and that develops new technologies that will help our state and beyond.

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West

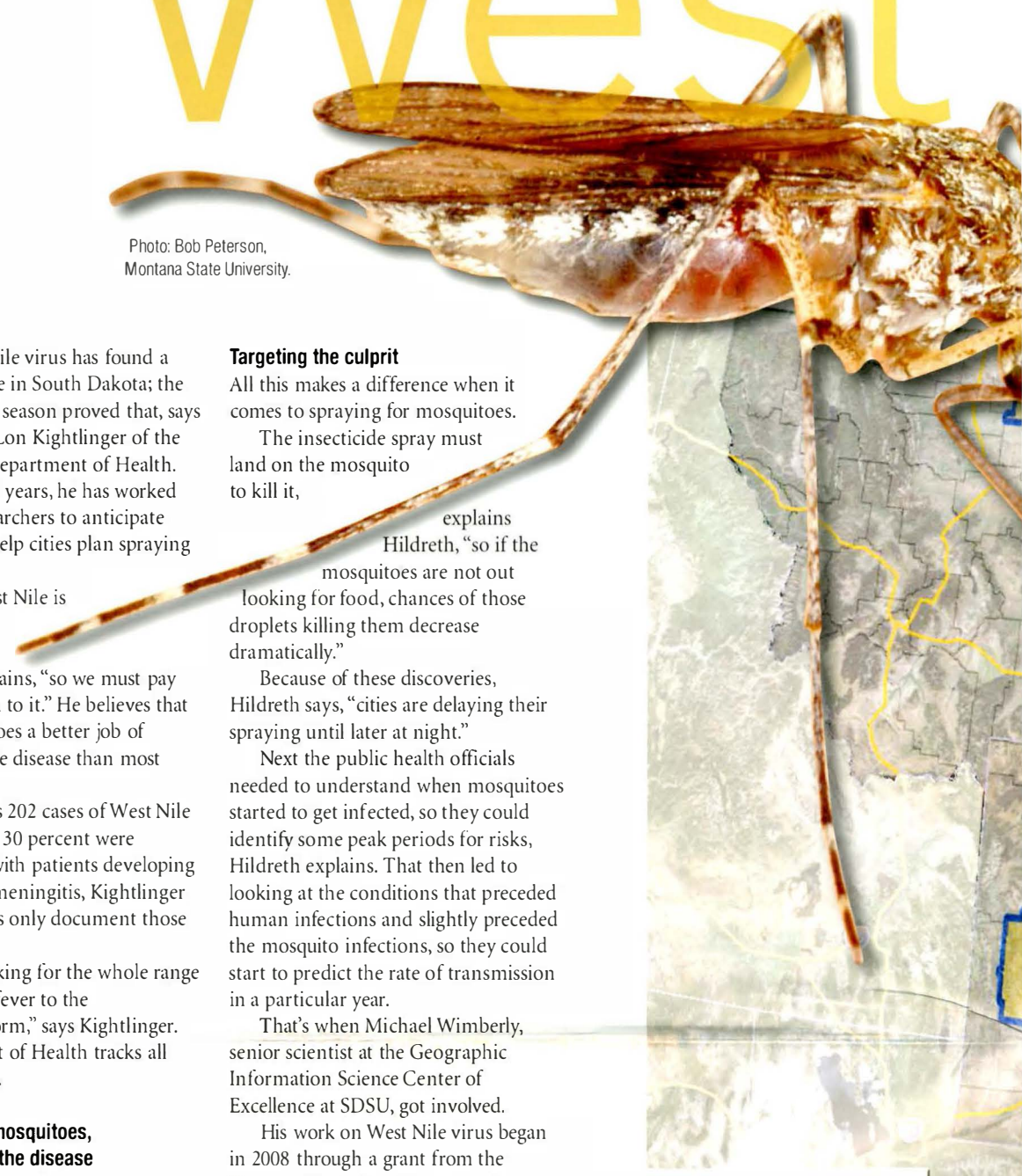


Photo: Bob Peterson, Montana State University.

West Nile virus has found a home in South Dakota; the 2012 season proved that, says epidemiologist Lon Kightlinger of the South Dakota Department of Health. Over the past 10 years, he has worked with SDSU researchers to anticipate outbreaks and help cities plan spraying tactics.

"We feel West Nile is pervasive in South Dakota," Kightlinger explains, "so we must pay special attention to it." He believes that South Dakota does a better job of documenting the disease than most states.

Of the state's 202 cases of West Nile this past season, 30 percent were neuroinvasive, with patients developing encephalitis or meningitis, Kightlinger says. Many states only document those cases.

"We are looking for the whole range from West Nile fever to the neuroinvasive form," says Kightlinger. The Department of Health tracks all confirmed cases.

Understanding mosquitoes, transmission of the disease

In order to deal with the disease, South Dakota public health officials needed to identify how it was transmitted, so they turned to professor of microbiology and biology Michael Hildreth.

To determine which mosquito carries the virus, Hildreth and his team of one master's student and three to four undergraduates set traps at sites across the state. Then he sent the captured insects to the public health laboratory in Pierre, where medical section supervisor Chris Carlson identified which mosquitoes were infected.

The main culprit turned out to be Culex tarsalis, Hildreth says.

Two species account for 90 percent of mosquitoes in South Dakota, Aedes vexans and Culex tursalis, according to Hildreth, and their breeding and feeding habits are very different.

Aedes vexans are the nuisance mosquitoes which hover in swarms at dusk, Hildreth says. Known as a flood water mosquito, Hildreth says, "it comes out fast in the spring of the year, and populations can increase very rapidly."

Culex tarsalis spend the winter as fertilized adults underground in burrows and females produce eggs only after getting their first blood meal, Hildreth explains. "It takes longer to get their cycle started."

Consequently the West Nile carriers come out later in the season, and they tend to bite later at night, between 10 p.m. and 2 a.m., Hildreth says.

Targeting the culprit

All this makes a difference when it comes to spraying for mosquitoes.

The insecticide spray must land on the mosquito to kill it, explains Hildreth, "so if the mosquitoes are not out looking for food, chances of those droplets killing them decrease dramatically."

Because of these discoveries, Hildreth says, "cities are delaying their spraying until later at night."

Next the public health officials needed to understand when mosquitoes started to get infected, so they could identify some peak periods for risks, Hildreth explains. That then led to looking at the conditions that preceded human infections and slightly preceded the mosquito infections, so they could start to predict the rate of transmission in a particular year.

That's when Michael Wimberly, senior scientist at the Geographic Information Science Center of Excellence at SDSU, got involved.

His work on West Nile virus began in 2008 through a grant from the National Institute of Allergy and Infectious Diseases. Wimberly analyzed satellite imaging data from 2000 to the present to build a store of information to begin predicting the risk of West Nile each year. Wimberly and his team perform statistical analyses using remote sensing data and then compare their results with Kightlinger's West Nile virus statistics.

"What we're doing is unique," says Wimberly. "We are trying to make a much tighter link from research to application."

As part of the project, associate professor Yi Liu from electrical engineering and computer science has developed software to manage the large amounts of data Wimberly uses. Then she created the framework to store, manage and display the maps that Wimberly and his team create. The maps are available at http://globalmonitoring.sdstate.edu/projects/eastweb/WestNileVirus.php.

Wimberly's team consists of one postdoc, one doctoral student, one master's student and two undergraduates, while Liu has four graduate students and six undergraduates working on the project.

This work can be useful to public health officials.

"It helps us get a handle earlier in the season on what may unfold," Kightlinger says, which then helps him plan ahead.

But he is cautious: "We are in a test modeling and learning process now, so we're not at the point where we can put the theory into practice in our communities."

"Giving a warning is easy, but what it comes down to is resources," Kightlinger says.

Small communities can strategically target their spraying before big events, such as a baseball tournament or the Fourth of July, Kightlinger explains, "which will lower the risk considerably."

Predicting outbreaks of the virus

Admittedly, the early warning system has not yet been perfected. Wimberly has only been making yearly predictions since 2010, but he's been right two out of three years.

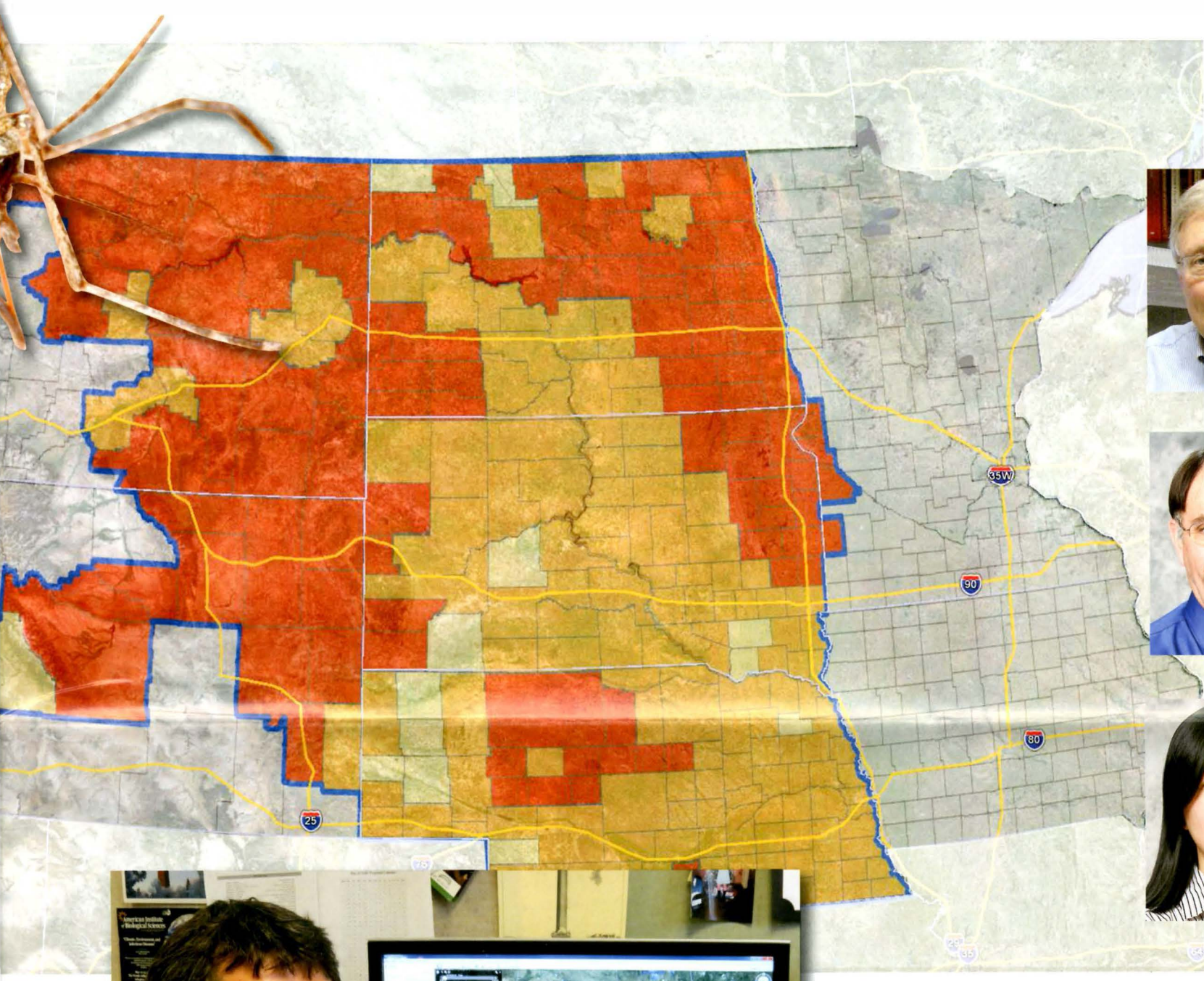
"Our research has shown that temperature is an extremely strong driver," he says.

Wimberly found associations between West Nile virus and temperature at two different times of the year. First, an earlier spring green up, like this past year with the warm April, gives the virus a longer amplification period, he says. "In general, mosquitos develop more rapidly, are more active and tend to bite more when it's hotter."

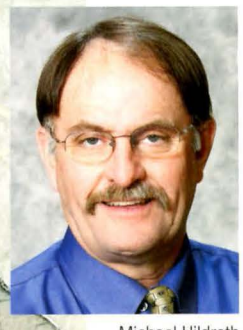
The virus originates in the bird population but is transmitted to humans through the mosquito, Wimberly explains. After the mosquito bites an infected bird, the virus must incubate in the mosquito before it can be transmitted to humans.

Nile

Researchers help public health officials combat West Nile virus



Lon Kightlinger

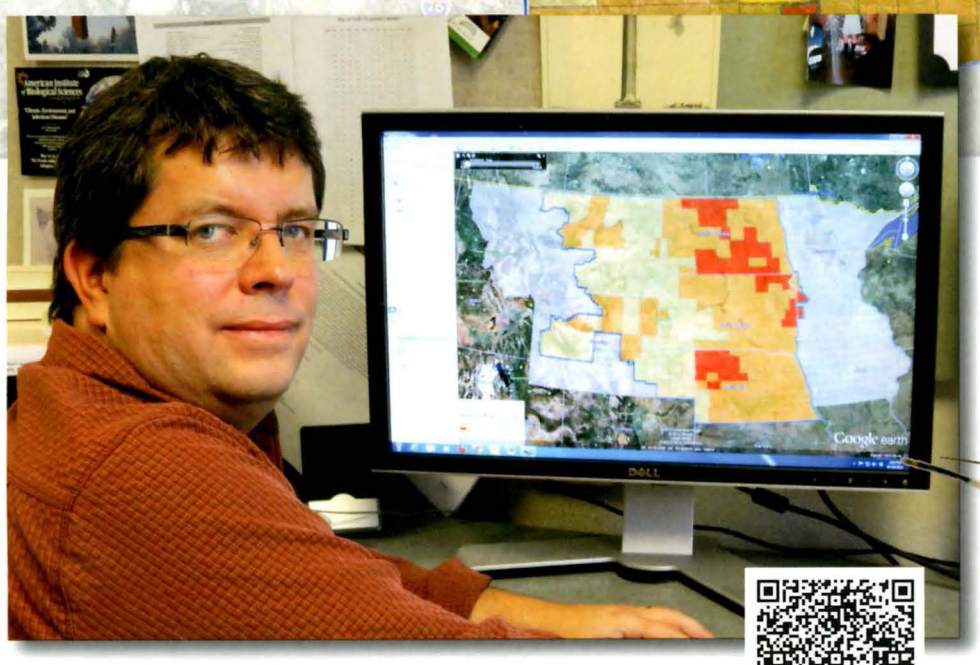


Michael Hildreth

Yi Liu

Each county in the Northern Plains is color-coded on this 2012 West Nile Virus Risk Forecast map based on the risk of human infections. Orange counties have the highest risk, gold ones, a medium risk and yellow, the lowest risk.

Test your West Nile virus knowledge with the quiz on the back page.



Professor Michael Wimberly pulls up another map that he and his colleagues have made available online at http://globalmonitoring.sdstate.edu/projects/eastweb/WestNileVirus.php.

This process is also temperature dependent, Wimberly says. During a warmer than normal summer, the mosquitoes become infectious more quickly.

When it comes to moisture and rainfall, Wimberly says, the relationship is complicated. As evidenced last year, lack of rainfall does not necessarily mean fewer West Nile cases.

The West Nile mosquito breeds in small water puddles, such as wheel ruts in a pasture or irrigated alfalfa. Wimberly calls it the "Goldilocks effect-the water needs to be just right."

Wimberly's next step will be to incorporate additional sources of environmental information to more precisely map the places where transmission risk is highest.

"Over time, we get better," Wimberly says of the ability to predict West Nile outbreaks. As a result, the public health community will begin to trust the forecasts. The ultimate goal is to provide useful and reliable information that can be applied to support West Nile virus prevention and control efforts.

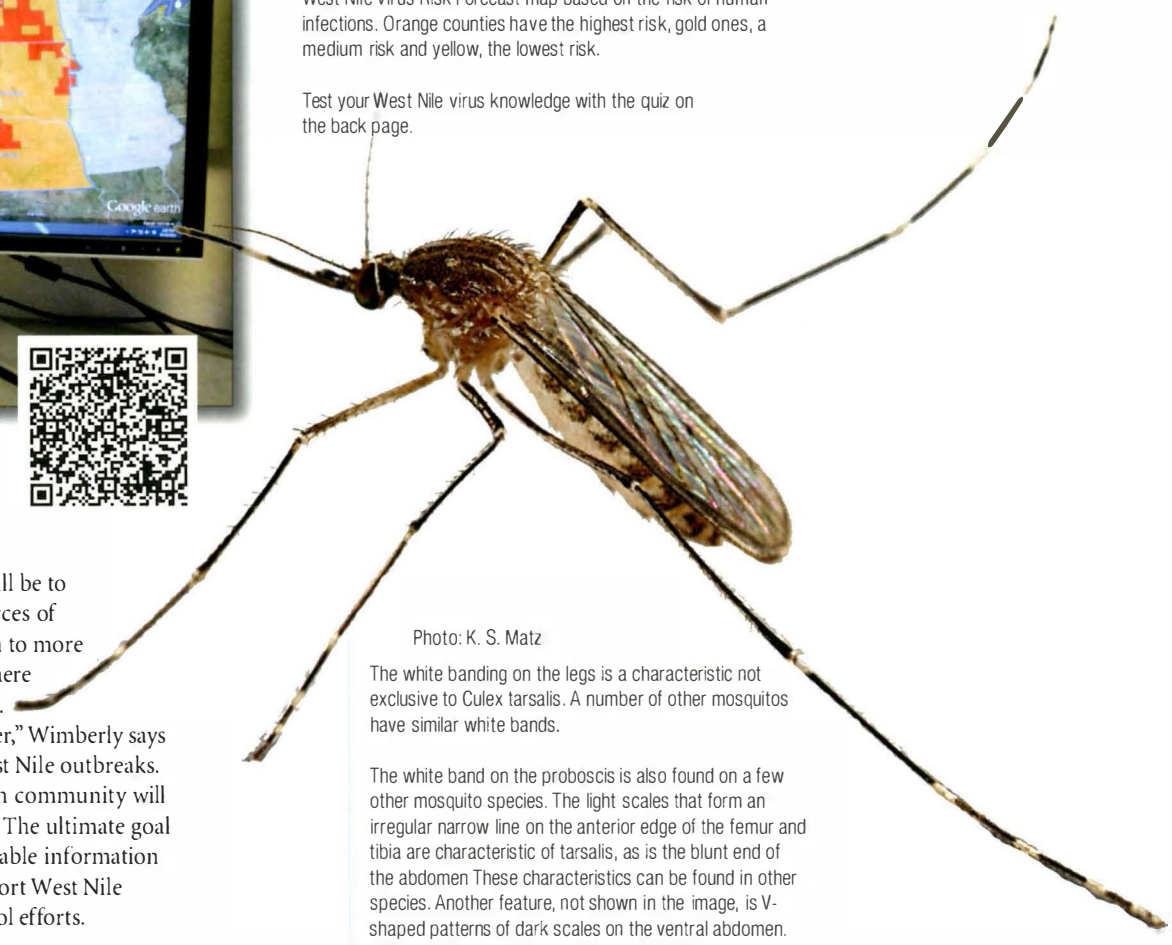


Photo: K. S. Matz

The white banding on the legs is a characteristic not exclusive to Culex tarsalis. A number of other mosquitos have similar white bands.

The white band on the proboscis is also found on a few other mosquito species. The light scales that form an irregular narrow line on the anterior edge of the femur and tibia are characteristic of tarsalis, as is the blunt end of the abdomen These characteristics can be found in other species. Another feature, not shown in the image, is V-shaped patterns of dark scales on the ventral abdomen. When all the characteristics appear together, the specimen will probably be Culex tarsalis.

Avera

Four collaborative research projects supported by Avera Health and by SDSU's colleges of pharmacy, education and human sciences, nursing, and arts and sciences will improve the health of South Dakotans. Each of the $20,000 awards is split equally between the SDSU and Avera researchers to support a one-year project.

Collaborative projects improve health-care techniques

Repairing damaged arteries

A compound developed and patented by Ron Utecht, a former SDSU professor and now scientific director at Avera Research Institute, may one day make stents obsolete.

Light-activated naphthalimide has been shown to bond tissues and link proteins in blood vessels to strengthen the artery walls after angioplasty, Utecht says. The first clinical trials of this application began in August.

Linhong Jing, assistant professor of chemistry and director of the core campus mass spectrometry facility at SDSU, and Karen Munger, director of basic research at Avera, want to find out how this compound works.

When blocked arteries are opened using balloon therapy called angioplasty, stents are sometimes used. "They do the job but are rigid and don't work well for arms and legs," says Munger.

A person's blood vessels are made of proteins and other materials, Munger explains. However, Jing says, when the arteries are diseased, some proteins expressed in the tissue may change. Finding out exactly which parts of the proteome, the entire set of proteins in tissue, are affected is the challenge. Jing will use a mass spectrometry technique which will allow her to analyze changes in protein concentrations and to characterize the structures of the proteins in the arteries at a molecular level, a field of study known as proteomics.

"Blood vessels are more than just hoses getting blood from one place to another," Munger says. "They are reactive and transfer nutrients and other physiological signals throughout the body."

The physiologist became interested in naphthalimide when she saw what it could do. "Blood vessels treated hold their shape but are not stiff," Munger says. "They feel normal and can be compressed, which is quite different from a stent."

When a balloon catheter is expanded to open a diseased artery, it stretches the blood vessel and damages it further, Munger explains. The naphthalimide compound stabilizes the artery, like a bandage or stitches do for a cut, and may allow the healing process to occur more rapidly.

"We know the compound cross-links with collagen to produce a scaffolding effect," Utecht says. But does it affect healing?

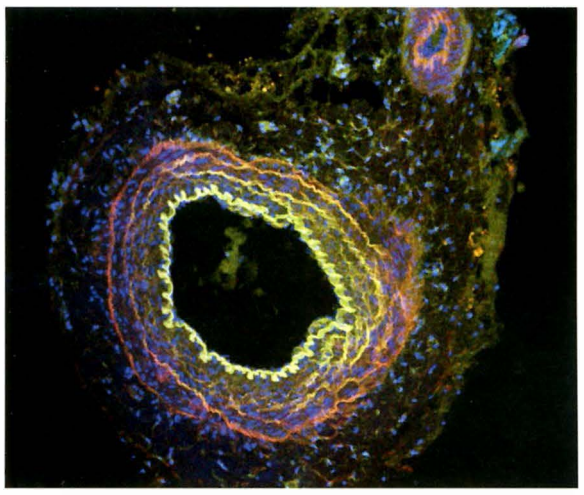
To figure out how naphthalimide works, Munger used blood vessels from rats and stretched them using a balloon. She then harvested the tissues and gave some of them to Jing, who will compare the protein profiles of normal and stretched artery tissues focusing on those proteins that have different concentrations.

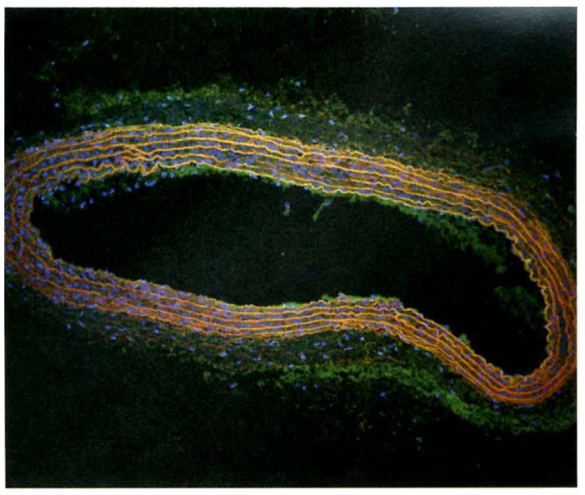
To find out which components of these proteins have changed, Jing will digest the long protein chains into peptides, the building blocks of proteins. Each peptide, Jing says, has a fragmentation pattern like a fingerprint. Then she will break apart the individual peptides into ions and identify them by using mass spectrometry. Comparing the features of the peptide fragments is like looking at all the ridges and patterns of a finger print, Jing says. She will determine which proteins' concentrations have changed in the damaged rat vessels.

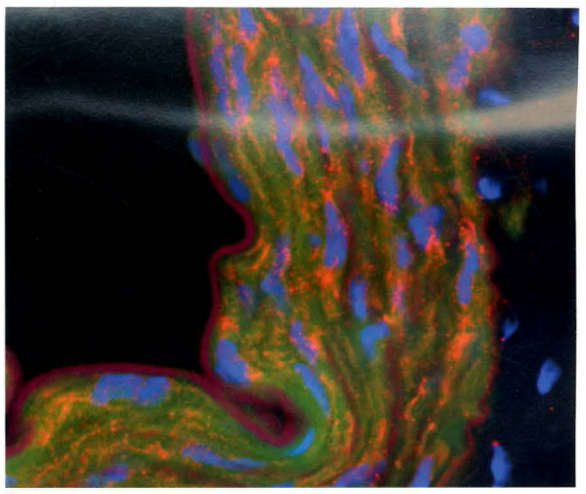
Then, Munger will do the balloon procedure and treat some arteries with the light-activated naphthalimide compound. After the vessels are given several weeks to recover, she will send them to Jing, who will analyze proteome in all three groups: unstretched or normal arteries, stretched to represent angioplasty, and stretched and treated with the naphthalimide compound. By comparing these samples, Jing will figure out which protein changes are associated with the disease and how naphthalimide affects the proteins in the arteries.

After this, Munger will analyze the types of proteins that are different and determine which ones are important in the disease process and after treatment with the light-activated naphthalimide compound. She will then use this information to further her research into the effects of light-activated naphthalimide compound on blood vessel damage, recovery and repair.

This information will help researchers develop new applications for the compound, Utecht says, "and even make a new compound that can do something better."







These microscope images of blood vessels allow researchers to examine the connective tissues which are made up of proteins, collagen in red and elastin in green, and help the vessel maintain its shape. The vessels, which are about the size of the wire in a paper clip, are taken from a rat. Scientists are studying how a compound called naphthalimide interacts with these proteins to help arteries recover after being stretched during balloon angioplasty. The image on the left is the cut end of an artery from the leg and the other two show first a cross section and then the outside of a neck artery. The blue dots are DNA in the nuclei of individual cells in the blood vessel.

Finding ways to reduce side effects of corticosteroids

Corticosteroids, such as prednisone and dexamethasone, reduce inflammation in patients dealing with ailments such as asthma and rheumatoid arthritis, but assistant professor of pharmaceutical sciences Teresa Seefeldt says, "these corticosteroids have a lot of side effects." One of these is increased blood sugar.

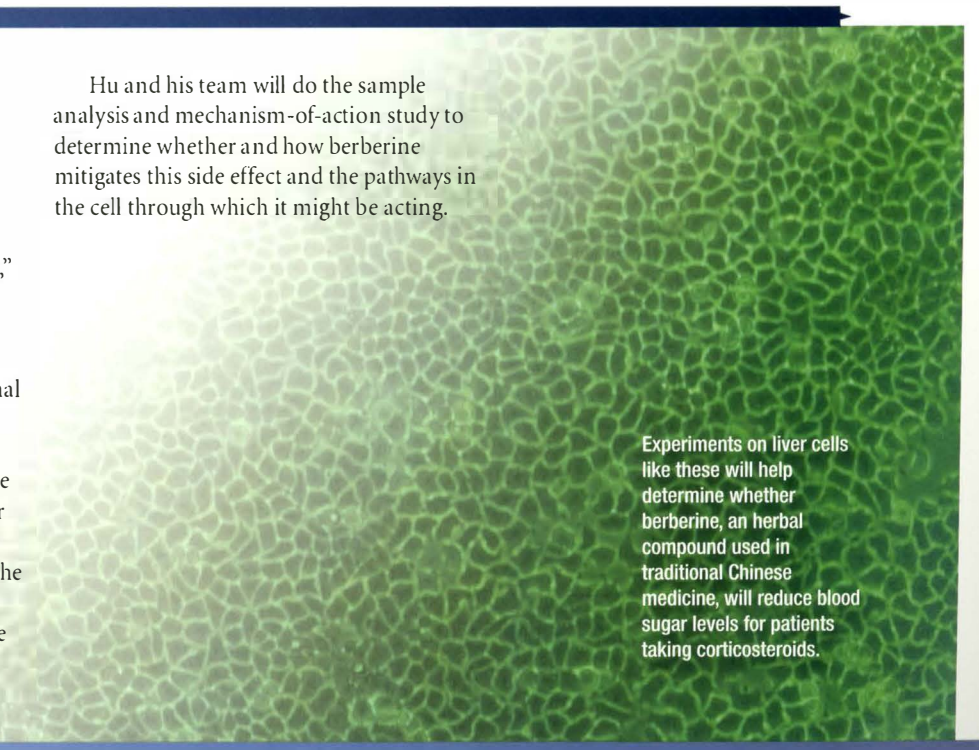
Seefeldt and Dr. Yueshan Hu of the Avera Institute for Human Genetics will explore whether an herbal compound called berberine, commonly used in traditional Chinese medicine, can lessen this side effect. Berberine is made from the roots of goldenseal, goldenthread, Oregon grape, barberry and tree turmeric.

Hu, who earned a medical degree in China and a doctorate in pharmaceutical sciences from SDSU, has worked on integrated Chinese and Western medicine. As a research assistant at SDSU and now as a research scientist at Avera, he evaluated berberine as a means to reduce weight gain, lipids, and sugar levels in mice and later in human subjects.

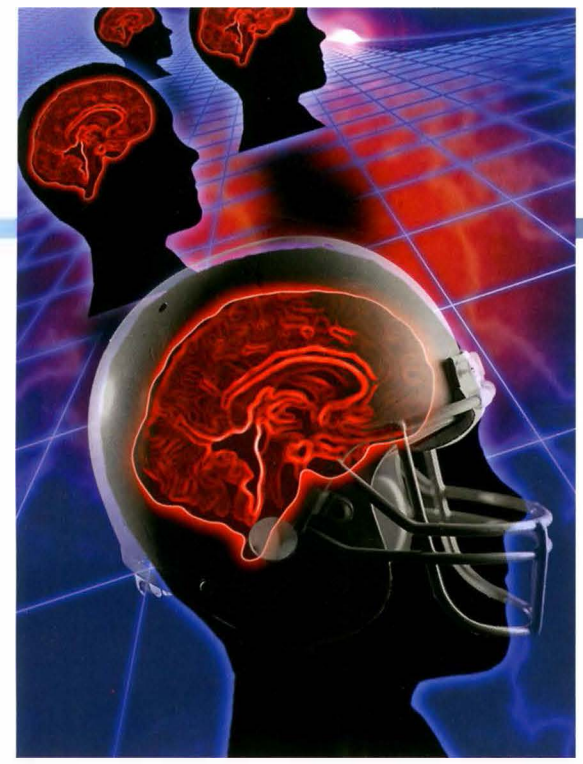
"Berberine can decrease lipid levels and weight in the human Caucasian population," he says. Based on these results, Seefeldt and Hu will explore whether it can do the same for patients on corticosteroids.

The team will use cell cultures and animal models to determine how combining a corticosteroid with berberine will affect glucose levels. One doctoral student and one undergraduate student are comparing sugar production in liver cells treated with the corticosteroid alone and a combination of the corticosteroid and berberine. The corticosteroid makes the liver produce more glucose, causing weight gain and diabetes.

Hu and his team will do the sample analysis and mechanism-of-action study to determine whether and how berberine mitigates this side effect and the pathways in the cell through which it might be acting.



Experiments on liver cells like these will help determine whether berberine, an herbal compound used in traditional Chinese medicine, will reduce blood sugar levels for patients taking corticosteroids.



Caring for young athletes with concussions

A 2011 survey of U.S. high schools with athletic trainers reported that 15 percent of sports-related injuries are concussions; for seven Brookings area schools, that figure is nearly 6 percent.

Since fall 2011, 70 high school athletes have sustained concussions, 29 of them last fall, according to Bernadette Olson, associate professor in health and nutritional sciences. She is working with Avera Sports Institute to describe the severity and monitor the outcomes of sports-related concussions, particularly in rural youth. The 2012 research funding will allow her to continue that work.

In 2011, South Dakota passed legislation setting concussion policy for high school athletics. Since 2009, more than 40 states have done so.

The law requires parents and students to learn to recognize the symptoms of concussion and know the procedures they must follow when an injury happens. Coaches must complete free online training through the Centers for Disease Control and Prevention.

In addition, any player who gets a head injury must be removed from play immediately and cannot return until a health-care professional declares the athlete to be symptom-free. The law applies to all schools who participate in South Dakota High School Athletic Association sports.

Complying with the law is especially challenging for small schools with limited resources which often do not employ an athletic trainer, Olson says. Responding to this need, she contacted school athletic directors in the area.

"They wanted to do even more for the kids," Olson says. They were interested in using a neuro-cognitive assessment tool called ImPACT designed to evaluate injured players. Olson told them she could help and would like to document the results. Thus began their mutually beneficial partnership.

Since fall 2011, Olson and her team have performed baseline, pre-injury evaluations on 1,200 athletes in Brookings, Arlington, Castlewood, Deubrook, Elkton, Estelline and Sioux Valley schools. All seventh through 12th graders who participate in interscholastic sports were included in the study.

Last fall through the grant with Avera, her team of one master's degree student and eight undergraduates expanded the testing to soccer and hockey players, sports not governed by the SDHSAA.

When an injury occurs, the athlete undergoes neuro-cognitive and balance tests, Olson says. Typical symptoms are headache, nausea and dizziness. In the first 24 to 72 hours, Olson says, "we expect them to not look good." Usually the athlete is kept home from school on bed rest.

Within two to three weeks, the symptoms usually subside. However, if they persist, Olson calls in Dr. Samuel Schimelpfenig, a pediatrician who is also board-certified in sports medicine at Avera's McGreevy Clinic.

"This is highly valuable work," Schimelpfenig says. He is seeing more patients with concussions, in part due to increased awareness about the symptoms and the need to be evaluated by a medical professional.

Because of the success of Olson's pilot program, Schimelpfenig says they are now writing a grant proposal that may expand this work to 20 schools within a 60-mile radius of Brookings, again targeting small schools where the resources are more limited.

In addition to comparing their results based on gender, Olson and her team will assess quality of life. "Can they tolerate school, take the test, or drive a car?" she says. "Our role is to make sure we're doing the testing, following it and describing it."

Eventually Olson would like to get more longitudinal data, looking at whether a concussion affects athletes 20 years down the road. This is a goal which Schimelpfenig says is worth pursuing, particularly since he's seeing more junior high athletes with concussions. "We do not know what the future holds for these young people."

Football and ice hockey put high school athletes at the highest risk for sustaining a concussion, despite their use of helmets.

Finding ways to help nurture preemies

Babies born four to s x weeks early look like normal infants, though just a bit smaller. But looks can be deceiving.

These late preterm babies, ranging in size from 4.4 pounds to more than 6 pounds, can experience health problems because their systems are not fully matured, says assistant professor of nursing Haifa Abou Samra. Of the 525,000 babies in the U. S. who are born early, 71 percent of them are from 34 weeks to less than 37 weeks gestation.

She and Avera Neonatal Intensive Care Unit Manager Janet Dutcher have begun a pilot feasibility study to investigate how snuggling skin-to-skin with their mothers can help these premature infants thrive. Dutcher, who serves on the Governor's Task Force on Infant Mortality, says approximately 11 percent of infants in South Dakota are born early; nearly three-fourths of those will be in the late-preterm category.

The practice, commonly called kangaroo care, began in underdeveloped countries where no neonatal care facilities are available. The baby is placed on the mother's bare chest immediately after delivery. "This is a natural means of helping the baby maintain its body temperature and physiological stability right after birth," Abou Samra says. In her study, the babies will be clad only in a diaper and placed on their mothers' chests with a blanket to secure them together.

Studies so far have documented the benefit of kangaroo care, recommending that snuggling sessions last 50 minutes, Abou Samra says, but they don't know the optimal frequency and number of sessions, weeks or months that mothers should use the technique to achieve this benefit.

In the study, one group of mothers will begin kangaroo care as soon as their infants are born, while another, *particularly those whose babies are transferred* from other hospitals, will start the care after the preemies arrive at the neonatal intensive care unit.

"We need scientific data to support decisions we make, whatever we suggest for families," Abou Samra says. She has two undergraduate students working on the project.

Abou Samra says she hopes to involve around 60 mother-baby pairs in the research, varying the level, frequency and length of time kangaroo care is used.

"Our role is to support the mother and to facilitate the natural bonding processes between mother and baby in this difficult environment," she says. The researchers and their teams will document the infants' physical development and their mothers' feelings and perceptions about their infants' vulnerability before and after the study is completed.

"Kangaroo care has the potential to improve outcomes for moms and babies," Dutcher says, "and will also promote breast-feeding."

A third portion of the study will look at the use of kangaroo care for mothers whose babies face a fragile outcome and unfortunately will not survive. The goal is to see if the skin-to-skin experience comforts babies and helps the mothers deal better with bereavement. "While we wish the outcome was different for those families, we *are committed to* providing them the best care possible," Abou Samra says. Again, the literature recommends this, but no scientific evidence has been gathered to support it. She hopes to provide this type of care for eight to 10 families.

"These parents are vulnerable and already stressed," says Abou Samra. "We need scientific data to support the decisions we make, so we can serve our population with confidence."

"And when time is of the essence, to do so with compassion," Dutcher adds.

This feasibility study will help the researchers perfect their procedures, strategies and protocol, Abou Samra says. Based on what they learn, they will seek national or private funding for a larger study.

Born at just over 36 weeks gestation, Rutledge Quinn snuggles skin-to-skin with his mother Jamie as part of the kangaroo care project. The Quinn family is from Ruthben, Iowa.

Oilseeds

South Dakota oilseeds, processing method may fuel Navy green fleet

Mustard

Camelina

Flax

Navy ships and airplanes may one day be powered by fuels made from oilseeds grown in the Midwest and a processing method developed by South Dakota State University researchers.

During the 2012 session, the South Dakota Legislature set aside $450,000 per year over the next three years to develop a "drop-in" equivalent fuel that can be mixed or alternated with petroleum products. The North Central Sun Grant Center at SDSU is providing an additional $390,000 of federal funds in support of an oil extraction project that was initiated by several SDSU investigators in late 2012. The center promotes collaboration to develop bio-based transportation fuels.

According to Daniel Scholl, director of the South Dakota Agricultural Experiment Station, this oilseeds initiative will take a comprehensive look at breeding, genetics, conversion, fuel characteristics and economics.

"It's really about opportunity and economic development for South Dakota, and what's attractive about it is that there's a customer waiting. That's why we wanted to grasp the opportunity," says Scholl.

Chris Tindal, director for operational energy for the Navy, has estimated that by 2020 the Navy will need 8 million barrels per year of alternative "green diesel" for surface ships and also 8 million barrels per year of jet fuel to run supersonic fighters, helicopters, logistical aircraft and generators.

"We seek to do the work that will have the greatest impact on South Dakota's potential production of bio-based jet fuels and that effectively leverages state funds," Scholl says.

All except $70,000 of first-year funds have been allocated, according to initiative director William Gibbons. During the initial phase, the researchers will examine a wide range of possible oilseed crops: flax, crambe, camelina, mustard, canola, field pennycress, safflower, sunflower and other rapeseed.

However, SDSU researchers have laid the groundwork through a prior Sun Grant project with North Dakota

After the oilseeds are milled, ethanol is added as a pretreatment. Three hours later, the mixture is run through an extruder to determine how much oil can be extracted.

State University begun in 2011 to determine the suitability and sustainability of various oilseed crops in the Dakotas and to assess the oil and fuel characteristics of the seeds.

Developing oilseed varieties

Since fall 2011, Extension oilseed specialist Kathy Grady and agronomist Thandiwe Nleya have been collaborating with fellow plant scientist Burton Johnson, of North Dakota State University, on a two-year Sun Grant project to identify potential feedstocks for an oilseed-based biofuels industry in the Dakotas.

Grady, Nleya and Johnson are looking at the viability of flax, canola, safflower, crambe, and brown mustard, according to the proposal, by planting these crops at selected sites in both states. South Dakota's plots are located in Brookings, Pierre, Wall and Bison.

During the last two years, dry conditions have hampered the fall germination of her winter camelina crop, Grady says. This resulted in harvesting problems due to different levels of maturity. In 2011, only 30 percent of the seeds came up in the fall, Grady explains, so the pods on the early plants had shattered before the rest of the plot was ready to harvest. "A more uniform time of emergence," she says, will result in a better outcome.

Field pennycress, which is a weed in small grains, has a desirable oil composition, she says, "but it has a lot of agronomic issues to be resolved through breeding to turn it into a viable crop."

Grady has been testing a new variety of canola that is reportedly more heat and drought tolerant, plus a more traditional canola variety; however, neither did well in Pierre and Brookings due to the challenges of the 2012 growing season, she says. "It was not a very good year for testing."

"The plots are small and the yields were not huge," she says, but she was able to harvest a few kilos of each crop species. These she delivered to professor Kasiviswanathan Muthukumarappan of the agricultural and biosystems engineering department, who will determine which seeds have the greater potential for oil and fuel production.

Determining oil and fuel characteristics

Muthukumarappan will experiment with methods to extract the oil from the seeds. In addition to oil yield, he will find methods to evaluate the quality of the oil produced, he says. Professor James Julson and assistant professors Lin Wei and Zhengrong Gu are also part of the agricultural and bioprocessing team's two-year project, which began Nov. 1.

The first step, Muthukumarappan explains, "is looking at the effect of different green solvents and trying to compare those results with hexane extraction," a commonly used method that uses a component of gasoline.

"Green solvents are truly natural and renewable," says doctoral student Parisa Fallahi, "and don't have any adverse effects on the environment." Fallahi will first look at ethanol as a model for extracting oil.

Then she will evaluate two organic compounds as solvents, d-limonene made from citrus fruits such as oranges and lemons, and alpha pinene made from pine trees. If she can get a high recovery of oil with these compounds, she says, this will benefit the environment and add value to these products.

Fallahi is testing two methods. The simplest one is to grind the seeds, soak them in ethanol for three hours and then extract the oil. The other uses a combination of ethanol as a solvent and extrusion, which applies heat and pressure to break down the seeds and release the oil. So far she has applied this technique to canola, flax and camelina seeds.

To conduct the initial research, a simple batch method is being used to process small amounts of the seed. Eventually Muthukumarappan and his team of one doctoral student and two part-time postdocs will develop a continuous processing method. Half of his $500,000 funding will go toward purchase of equipment, including a pilot-scale extruder. (See related story.) The rest will support staff, materials, supplies and some travel.

Muthukumarappan's goal is to identify which seeds produce the largest quantity and highest quality of oil. Determining the most efficient means of extracting the oil is part of that task.

Once the group has developed a continuous method of extracting the oil, the bioprocessing team will look for an industrial partner to convert the oil into jet fuel.

Making crops profitable for farmers

Another challenge in this process will be to convince farmers that they can make a profit on oilseed crops. That's where associate professor Bashir Qasmi comes in. "Profitability leads to production," the economist says.

To determine the crop potential, Qasmi must first look at how oilseed production in Montana and the Dakotas has changed in the last 10 years. Qasmi says that combined acreages of sunflower, safflower, canola, flax and mustard in these states have decreased by 30 percent. Montana and North Dakota have experienced large decreases, while South Dakota had a slight increase.

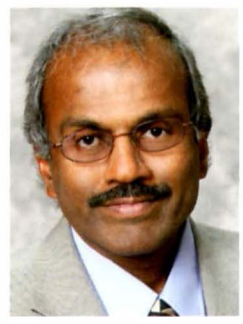
Qasmi needs to find out why these acreages have decreased. To do this, he must analyze the trends in relevant crop-reporting districts in each state and investigate comparative profitability of different oilseeds relative to other crops grown in the area. He also needs to collect the data on soil and weather conditions in the crop-reporting districts which can impact oilseed production.

During the second year of the initiative, the plant scientists and bioprocessing engineers plan to focus on two or three specific oilseed crops they deem most suitable for the area. Based on these recommendations, Qasmi can determine how these oilseeds will fit into the farmers' crop rotation plans and then estimate production potential.

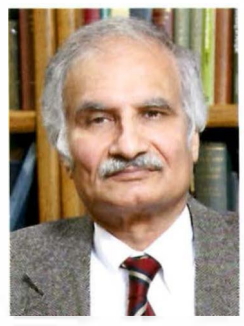
"Only then can we address issues relating to supply chain and processing locations," Qasmi says.

He anticipates that increasing yields and profitability will be needed to motivate farmers to plant oilseed crops.

Kathy Grady



Kasiviswanathan Muthukumarappan



Bashir Qasmi

Each of these vials contains oil extracted using a different method. The goal is to determine which seeds and extraction method will produce the largest quantity and highest quality oil.

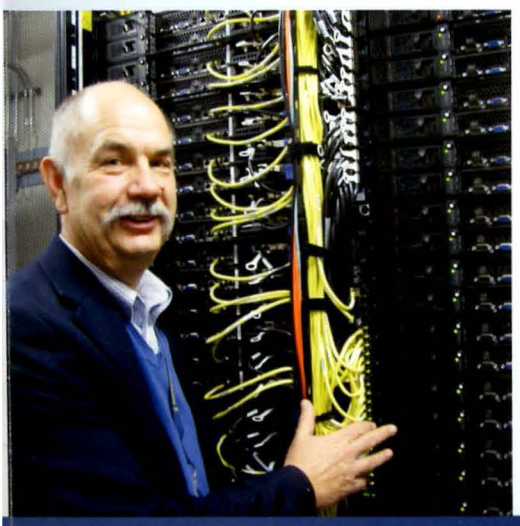
Equipment, computational power integral to research

Two major pieces of equipment and an increase in computing power will improve SDSU's research capabilities. These purchases were made possible through federal, state and industry funding.

With $500,000 from the South Dakota Board of Regents through a one-time 2012 legislative allocation, SDSU has purchased a pilot-scale extruder and scanning probe microscopy system which will support work across many disciplines.

The extruder will help researchers scale up the processing of oilseeds for use as drop-in fuel and soybean meal as a protein component in commercial fish food, just for starters. Because of this, the South Dakota Oilseeds Initiative contributed $100,000 and the South Dakota Soybean Council, $110,000, so that only $289,000 from regent funding was needed to pay for the system. Professor William Gibbons of biology and microbiology says he expects delivery of the system this spring.

Another $210,000 of the regents' funds have been used for a scanning probe microscopy system and other materials characterization instruments, such as upgrading an X-ray diffraction system purchased with a National Science Foundation grant.



New computing power comes with a smaller footprint but faster data analysis, explains Michael Adelaine, vice president for information technology.

Associate professor Qi Hua Fan of electrical engineering and computer science explains that the microscopy system has many applications. The system can analyze a broad variety of inorganic and organic materials at nanoscale, he says. These functions are valuable not only to electrical engineers developing electronic materials and devices, but to chemists studying liquid solutions, biologists evaluating tissue samples, and mechanical scientists analyzing deformation.

When the system arrives in March 2013, Fan plans to invite researchers from a wide range of departments to attend the training.

In addition, Michael Adelaine, vice president for information technology, says researchers can now analyze more data faster through new high-power computing. The $900,000 system was purchased through local funds made available by a National Science Foundation grant.

A pharmacist doing cancer research can model chemicals in 90 minutes that once took two months to do, Adelaine says. A chemist analyzing proteins can look at 90,000 atom interactions in a day and a half.

In addition to the time savings, Adelaine says the researchers also have more modeling programs and better access to them.

"Students can stay focused," says assistant professor Mahdi Farrokh Baroughi, thanks to the new system. They get results in less than a day that once took nearly a month to produce. His electrical engineering students are accessing MATLAB remotely to develop their own simulation models and then are fitting them with experiments to look at solar cell materials on an atomic level.

These investments will help researchers work more quickly to develop renewable energy, add value to South Dakota products, and strengthen the state's economy.

Undergraduate researchers solve problems, become experts

One undergraduate researcher sums up his experience like this: "Here is the system, this is the problem, this is our goal . . . now go for it." Brian Lawburgh and his twin brother Jim, both seniors in mechanical engineering from Hendricks, Minn., have been working on a renewable energy project to turn sawdust into jet fuel for nearly three years. "It's been a huge lesson in problem-solving," says Brian.

Sophomore microbiology major Kate Kondratuk of Brookings agrees. Before she can figure out how bovine viral diarrhea virus neutralizes the cow's immune cells, she says, "I have to work through a lot of kinks."

Microbiology undergraduate becomes expert on immune cells

Last year Kate received an American Society of Microbiology Undergraduate Research Fellowship to study how immune cells called neutrophils defend the cow's body against the virus and how the virus then neutralizes them. Her fellowship provides a $4,000 stipend for her research work, a two-year student membership and a trip to the society's presentation institute and general meeting in May.

Bovine viral diarrhea virus is actually an immune-suppressive disease, explains her adviser, professor Chris Chase of the veterinary and biomedical sciences department. "White blood cell count goes down, and the animal becomes susceptible to other infections." Pneumonia and respiratory diseases are the most common outcomes.

When an infection invades the cow's body, neutrophils are released into the bloodstream and rush to the site of the infection to eliminate it quickly, Chase explains. Once they spring into action, these immune cells live for only six hours.

Since the 1970s, very little research has been done on neutrophils, Chase says, simply because working with them is so difficult.

"They die just like that," Kate says as she snaps her fingers.

She has figured out that if the cow has been running or stressed before the blood is drawn, the immune cells are more likely to clump. "They are very delicate," she says.

She also puts the tubes of blood on ice as soon as they are drawn and centrifuges them at 4 degrees Celsius.

"Cold is the key to keep them from getting activated," Kate says. Once activated, neutrophils die quickly, and she must wait until more blood can be collected.

Despite the challenges of working with these cells, Kate says, "I think they are cool. The neutrophils eat the bacteria and spit out extracellular traps that catch and kill foreign bodies."

"This is not a trivial project," her adviser says, but Kate has become an expert on the subject. She has developed her own support network collaborating with the leading authority on neutrophils at the Agricultural Research Service National Animal Disease Center of the U.S. Department of Agriculture at Ames, Iowa, and a group of experts at the University of Wisconsin.

"I love the immune system, particularly the complex interactions of pathogens and immune systems," Kate says.

Although she finished her fellowship in December, Kate estimates that what she wants to accomplish on the neutrophil project will take the rest of her undergraduate career. She is continuing her research through the Honors College as an independent study project.

Aspiring mechanical engineers advance biofuels project

Like Kate, Brian and Jim are in the honors program and were sophomores when they began work in the biofuels laboratory.

The twins worked on two types of pyrolysis reactors designed to convert plant material into bio-oil, which must then be further processed to make biofuel, explains mechanical engineering professor Alex Moutsoglou.

The biofuels laboratory began as a part of a Department of Defense project to use lignin from prairie cordgrass, a native North America species, to make a bio-based jet fuel. The pyrolysis work was done in collaboration with an SDSU partner, the Biomass Conversion Division of American Science and Technology, an engineering firm with offices in Brookings and Wausau, Wis.

The Lawburghs worked on solving problems with the fluidized bed reactor and the cyclonic reactor, Moutsoglou explains.

Neither had any experience in alternative energy, but Brian says, "We jumped right in and started learning."

Once they developed an understanding of the process, they began the task of getting the systems to produce bio-oil. Because the reactors are complicated systems, Jim explains, "you have to start at the beginning and work your way through the whole system."

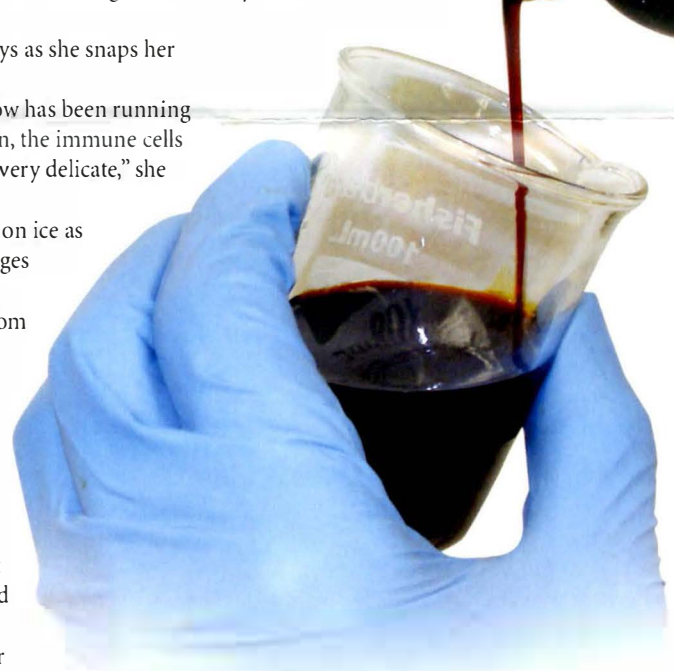


Photo courtesy of American Science and Technology, Biomass Conversion Division

But Moutsoglou believes they can do it: "They are problem-solvers."

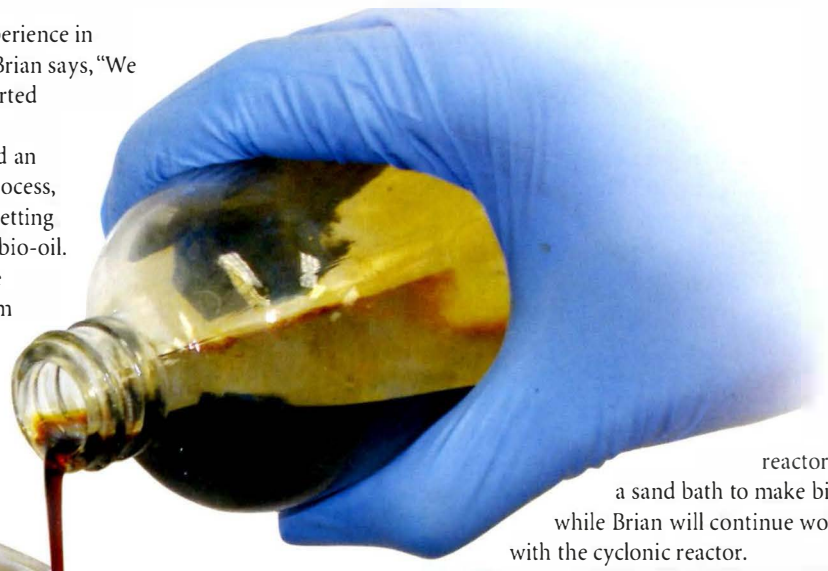
To check the flow in the cyclonic reactor, the Lawburghs modified the system making the T-fittings transparent to help detect any problems, Jim says.

Then they had students from the SDSU Materials Evaluation and Testing Laboratory take high-speed video of sawdust entering the reactor, Brian says, "to slow down those particles that were just a blur." This allowed them to determine how long it takes for the sawdust to go through the reactor and whether the circulating particles were touching the sides of the reactor.

The sawdust heats up through contact with the hot wall, Moutsoglou explains. The high-speed video confirmed that this was happening.

The Lawburghs also used the video to pinpoint other changes, including improving their heating method. They're still discussing whether the system is purging all the air out of the hopper. The process must take place in the absence of air, Jim explains.

Each of the twins received a $2,500 Joseph Nelson Scholarship for original scholarly work through the Sun Grant Initiative. Jim will use a stainless steel batch reactor and a sand bath to make bio-oil, while Brian will continue working with the cyclonic reactor.



Research fuels creativity, professional development

All three Honors College researchers believe their projects have given them skills they could not develop in the classroom.

"Undergraduate research is one of the highest impact educational activities," says Tim Nichols, dean of the Honors College. "It makes a positive difference in their intellectual development, problem-solving ability, communication skills and their ability to work both in groups and independently."

Though Kate, Jim and Brian will complete their research projects as part of their honors program requirements, Nichols says, the opportunities to do undergraduate research work are not limited to honors students.

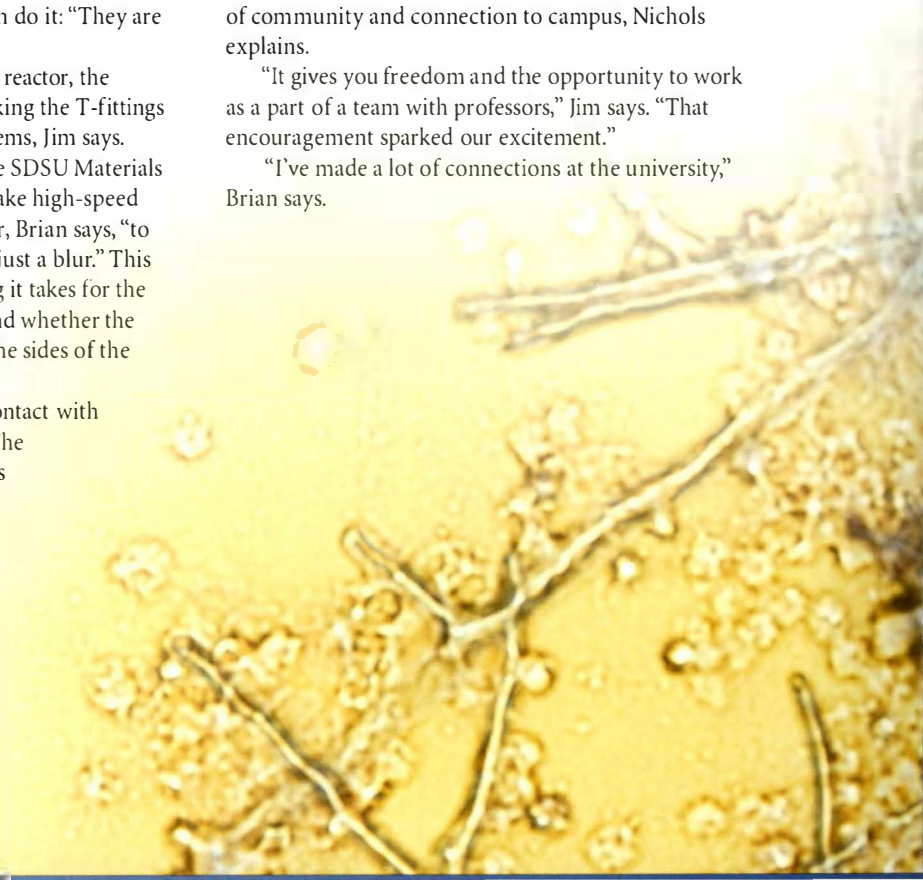
"Research work not only strengthens their academic knowledge about the research topic," Nichols says, "but builds broader skills which are important for success."

Jim agrees, "Doing research opens up your mind to be creative, challenges you to solve problems the best way that works for you."

Undergraduate research increases the students' sense of community and connection to campus, Nichols explains.

"It gives you freedom and the opportunity to work as a part of a team with professors," Jim says. "That encouragement sparked our excitement."

"I've made a lot of connections at the university," Brian says.



Both are looking at graduate schools and hope to stay in the Midwest.

"I feel like I've had an amazing opportunity to start my career already through undergraduate research," says Kate, who plans on getting her doctorate in immunology and infectious diseases first and then a medical degree.

As undergraduates, these students have formed a foundation for the skills it takes to succeed in a graduate program, Nichols explains.

"They have already had exposure in exploring the literature, in developing skills with research methods, in setting up an experiment and working it through from data analysis to conclusions to dissemination."

Professor William Gibbons of biology and microbiology recalls the experience of Andrea Opp, who worked for three years as an undergraduate in his lab. She went on to earn a master's in microbiology in 2000 and was hired at Cargill. When she and a dozen other new employees, many with Ivy League degrees, were given tasks in the research lab, Gibbons says, "Andrea was making progress, while they were stuck on simple things like how to calibrate a pH meter." Because of the leadership role she took mentoring the others, she soon had her own projects, one of which resulted in a patent.

When students "go for it" as Brian Lawburgh suggests, they can be assured that undergraduate research will teach them important lessons they can use for a lifetime, whether they are looking for employment or going on to graduate school.

Senior Jim Lawburgh carefully lowers a stainless steel tube into a silica sand bath heated to at least 500 degrees Celsius. For his Sun Grant research project, he will use this batch reactor to convert different materials into bio-oil, shown on opposite page, and then evaluate the quality of that oil.

Using the cyclonic reactor, senior Brian Lawburgh explains how sawdust flows through the hopper and into the reactor through a tiny tube. The carrier gas then forces the material in a circular motion so that it makes contact with the hot walls of the reactor.

Learning to culture immune cells is tricky business. Sophomore Kate Kondratuk checks the containers that are part of her experimental setup for plating immune cells called neutrophils, shown left.

SANFORD® HEALTH

Sanford Research and its partners awarded a $13.5 million grant from the National Institute on Minority Health and Disparities to bring together the tribal communities and health-care professionals

Organ donation key to helping Native Americans on dialysis

Twelve years ago when Margo Heinert, right, gave a kidney to her daughter-in-law Gena Heinert, it changed both of their lives. The two women share their story to help spread the word about living kidney donation and the rewards it brings.

Nancy Fahrenwald

Native Americans are the most likely of all the world's populations to develop diabetes. Once diagnosed, they are 3.5 times more likely to experience kidney failure than white Americans, according to the Journal of the American Medical Association.

SDSU's director of nursing research hopes to improve the outlook for native people and their families facing kidney disease.

Professor Nancy Fahrenwald received funding for a five-year, $1.32 million research project to promote kidney donation among three tribes in South Dakota.

She will work with co-investigators Howard Wey, an expert on biostatistics, and Robin Peterson-Lund, a family nurse practitioner and member of the Oglala Lakota tribe. Fahrenwald's team will also include one campus-based and two tribal coordinators, a doctoral student and two or three undergraduates. Research funds will cover personnel, travel, program development and data collection.

The project is part of a $13.5 million grant from the National Institute on Minority Health and Disparities awarded to Sanford Research and its partners to bring together the tribal communities and health-care professionals

The award will establish a Collaborative Research Center for American Indian Health in Sioux Falls under the leadership of Amy Elliott, director of the Center for Health Outcomes and Prevention Research at Sanford Research/USD. The center will work with tribal leaders to educate and engage the communities in ways that will improve health care for American Indians in South Dakota, North Dakota and Minnesota.

Initially, the center's research component will target three areas: pediatric asthma, emergency room usage and kidney disease.

Kidney failure common among tribes

"Everybody knows somebody who has renal failure because of diabetes, who needs a kidney, got a kidney, or died while waiting for a kidney," Fahrenwald says of her 10 years of research among Native American populations in the Midwest.

The best practice for kidney donation is getting evaluated when the kidneys begin to fail and being placed on the transplant list or identifying a living donor, Fahrenwald explains. Her project focuses on educating Native Americans about becoming kidney donors.

"This project will find the best ways to help open up the lines of communication for patients and families to talk about the options that are available to them. And those options can be life-saving," says Elliott, the new center's leader.

However, organ donation can be a difficult subject for Native Americans.

As a public health nurse, Karla Abbott, who is a member of the Cheyenne River Lakota Sioux tribe, has approached Native American families facing a loved one's death about the possibility of organ donation.

Native Americans must reconcile their beliefs about keeping the body intact for the afterlife with their culture's emphasis on generosity, says Abbott, who teaches nursing at Augustana College.

Abbott has served on the advisory boards for two of Fahrenwald's projects.

Beginning in 2003, Fahrenwald was co-leader of teams on two research projects aimed at encouraging American Indians on Pine Ridge, Rosebud, Cheyenne River and Sisseton-Wahpeton reservations to become organ and tissue donors. A Native American public relations agency helped the researchers design posters, brochures and videos to deliver their message.

Storytelling delivers message effectively

Through this work, Fahrenwald has learned that the most effective means of communication has been the oral tradition of storytelling. To reach students at tribal colleges in South Dakota, North Dakota, Minnesota and Montana, she used videos featuring kidney dialysis patients.

"One of the participants died while making the video," says Fahrenwald. "This speaks to how real and critical these issues are."

Some of her most powerful advocates are those who have survived to tell their stories. One of those is Gena Heinert, a 36-year-old member of the Rosebud Sioux tribe from Mission.

"I grew up with a lot of kidney infections," Gena says. By the time she was diagnosed with glomerulonephritis, a condition marked by inflammation of the tiny blood vessels of the kidneys, the damage was done.

But Gena is one of the lucky ones.

Mother-in-law gives gift of life

On Sept. 19, 2001, her 50-year-old mother-in-law, Margo Heinert, donated her kidney to 25-year-old Gena, who had been on dialysis for nearly seven years.

An article in a church newsletter prompted Margo to check into donating a kidney. After doing blood work at a local clinic, the two women went to Mayo Clinic for four days of intensive testing.

Before the surgery, Gena was more concerned for Margo than herself, but the doctors were amazed at how healthy Margo was at 50 and still is.

"I've been sicker with the flu," Margo says of her recovery.

The transplant went smoothly for both women.

"My kidney went to work on her right on the table," Margo says. She returned to work four days after the surgery. Gena went home after two and a half weeks in the hospital, when the usual stay is four to six weeks.

Both women know that their experience is the exception, especially among Native Americans, but as Margo says, "we want to help spread the word about this important topic."

Fahrenwald says, "Their story reflects not only the content of what is important to know about kidney failure, living kidney donation, and living kidney transplant, it personalizes it in such a way that people who hear the story will relate to the information on a personal level."

It's through powerful stories like the Heinerts' that Fahrenwald wants Native Americans and their families to learn about the life-saving options available for those dealing with kidney disease.

Researchers meet challenge of increased competition

2012 measurements

In an increasingly competitive environment, South Dakota State University researchers are meeting the challenge to secure funding for research and scholarship activities. This ability to compete in an era of reduced federal appropriations demonstrates improvements in the quality of the proposals and the innovative work done here.

With nearly $70 million in total FY2011 expenditures, SDSU ranked 175th among academic research institutions (Figs. 1 and 5).

SDSU researchers received greater funding this year from two federal agencies (Fig. 2). National Science Foundation grants increased by 20.7 percent from $7.01 million in FY2011 to $8.47 million in FY2012; whereas, NASA expenditures experienced a 13.7 percent rise, from $2.97 million to $3.3 million.

Furthermore, investment in research from the state of South Dakota and collaboration with private industries has led to a 11.4 percent increase in nonfederal funding, from $8.1 million in FY2011 to $9 million in FY2012. The South Dakota Legislature, the South Dakota Board of Regents and the Governor's Office of Economic Development have provided opportunities to expand our capabilities and launch new areas of research. In recent years, private industry has partnered with our scientists to develop value-added products and to improve the outlook for local industries.

In looking at the distribution among SDSU colleges, the College of Agriculture and Biological Sciences, which traditionally accounts for 30 percent of research expenditures, has maintained a high level of performance. In addition, Arts and Sciences experienced a 16.8 percent increase from $2.5 million to $2.9 million, and Education and Human Sciences expenditures increased by 5.8 percent from $3.78 million to $4 million (Fig. 3).

These research projects draw increasing numbers of graduate students to campus. The number of Ph.D. students increased again this year, and a record number of doctoral degrees were conferred in 2012 (Fig. 6).

SDSU researchers acknowledge the need to protect inventions and innovation, as intellectual property disclosures in 2012 kept up with the rapid pace set in 2011 (Fig 7). The licensing and commercialization of these new innovations then create new businesses and industries, expand job opportunities and improve the economic outlook for the state.

Fig. 1 Expenditures and Awards from Grants and Contracts, FY2004-2012

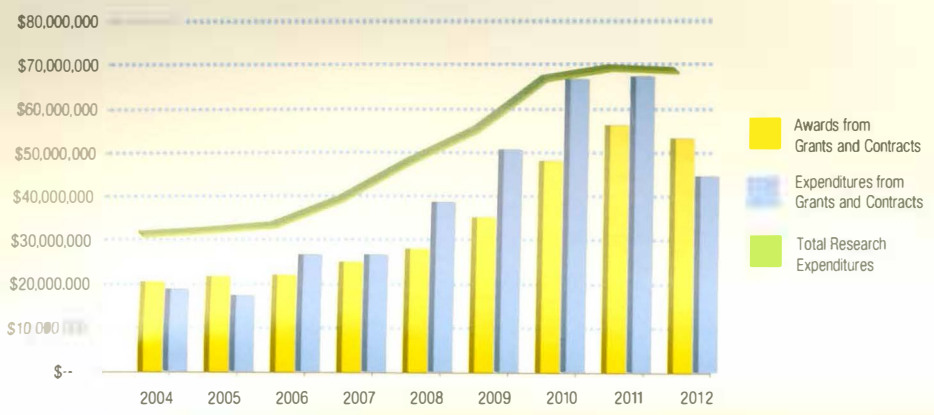


Fig. 2 FY2012 Grants and Contract Expenditures by Funding Source

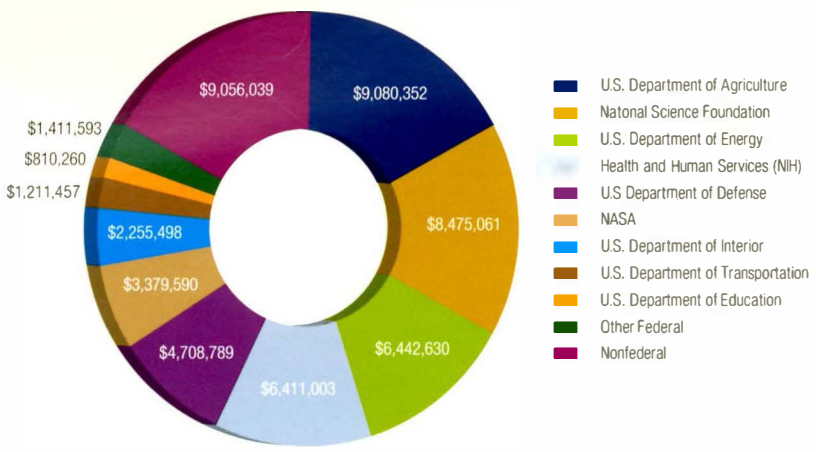


Fig. 3 FY2012 Grants and Contract Expenditures by Administrative Unit

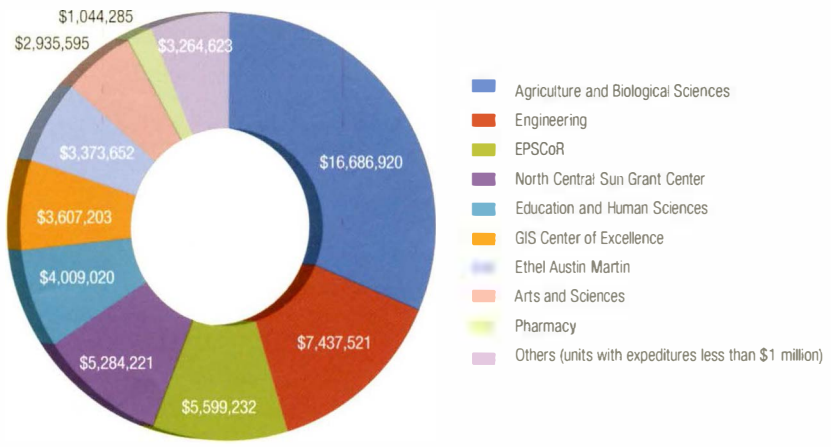


Fig. 4 Proposals Submitted and Awarded, FY2004-2012

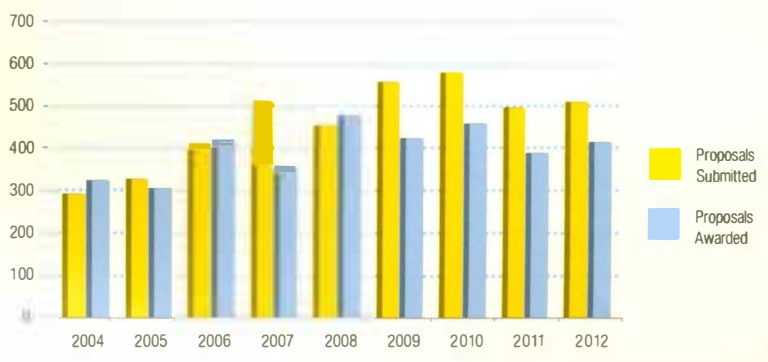
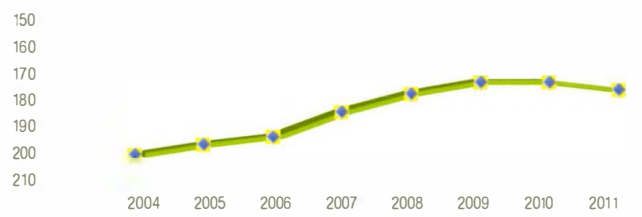


Fig. 5 Ranking Among Academic Research Institutions, 2004-2011



Source: National Science Foundation

Fig. 6 Ph.D. Program Enrollment and Degree Completions, 2007-2012

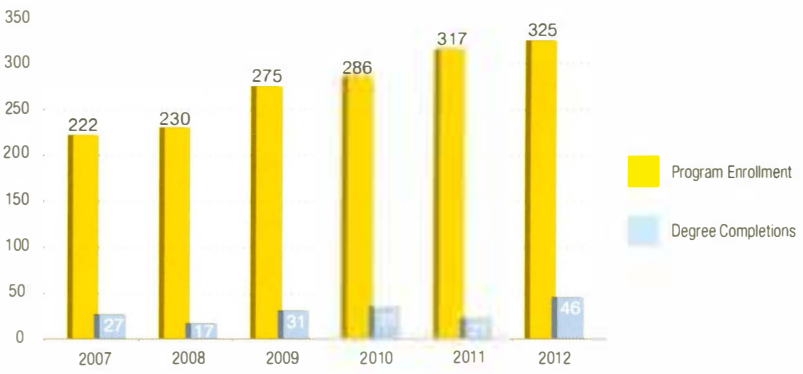
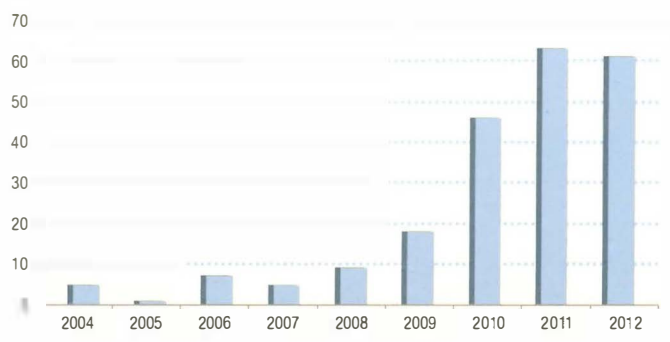


Fig.7 Intellectual Property Disclosures, FY2004-2012



Research Administration

SDSU Research Leadership

South Dakota State University
OFFICE OF THE VICE PRESIDENT FOR RESEARCH
SDSU, Box 2201
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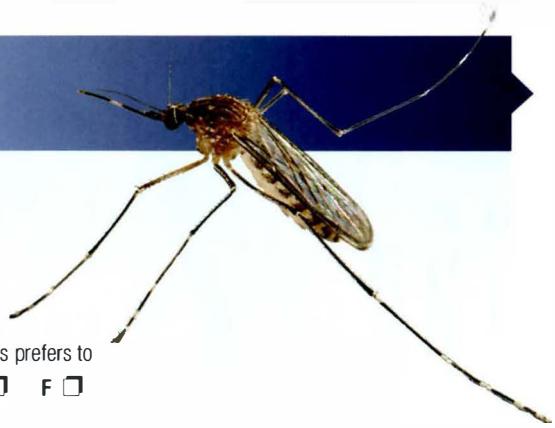
Quiz



Test your knowledge about

West Nile virus

True or False?

1. The mosquito that carries West Nile virus prefers to feed on birds rather than humans. **T** ❒ **F** ❒
2. West Nile virus can make birds sick, but it does not kill them. **T** ❒ **F** ❒
3. Horses, dogs, pigs and cows can become infected with West Nile virus. **T** ❒ **F** ❒
4. Stay away from the large swarms of mosquitoes at dusk, and you'll be safe from West Nile. **T** ❒ **F** ❒
5. The fewer mosquitoes we have, the fewer people will get West Nile virus. **T** ❒ **F** ❒
6. The earlier it gets warm and the longer it stays that way, the greater the chance for West Nile virus outbreaks. **T** ❒ **F** ❒
7. West Nile mosquitoes cannot survive in a drought. **T** ❒ **F** ❒
8. How the West Nile virus amplifies in the bird population is well documented. **T** ❒ **F** ❒
9. The West Nile-carrying mosquito spends the winter in burrows. **T** ❒ **F** ❒
10. The more mosquitoes we kill with spraying programs, the less West Nile virus will affect us. **T** ❒ **F** ❒
11. Nearly 80 percent of the people who contract West Nile virus never have any symptoms. **T** ❒ **F** ❒
12. South Dakotans run about the same risk as people in Minnesota and Iowa of contracting West Nile virus. **T** ❒ **F** ❒

Answers:
1. T
2. F
3. T
4. F
5. F
6. T
7. F
8. F
9. T
10. F
11. T
12. F

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